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(54) Title: ZINC(II) COMPLEXES AND METHODS RELATED THERETO

(57) Abstract

Zinc(II) complexes and methods relating thereto are disclosed. The zinc(II) complexes comprise a zinc(II) ion complexed by a multi-dentate ligand. Methods of this invention include the use of the zinc(II) complexes as anti-viral agents and/or as anti-inflammatory agents. Methods of this invention also include inhibition of viral infection, as well as inhibiting transmission of sexually transmitted diseases. Exemplary zinc(II) complexes include zinc(II):neocuproine (2:1) and zinc(II):bathocuproine disulfonic acid (2:1), including pharmaceutically acceptable salts thereof.

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Description

ZINC(II) COMPLEXES AND METHODS RELATED THERETO

Technical Field

This invention is generally directed to zinc(II) complexes and methods relating to the use thereof and, more specifically, to zinc(II) complexed by a multi-dentate ligand.

Background of the Invention

Zinc is found in both plants and animals and over 100 zinc-containing proteins and enzymes have been identified (Chaney, Textbook of Biochemistry with Clinical Correlations, pp. 1115-1147, Devlin (ed.), New York, New York, Wiley-Liss, 1992). Examples of zinc-containing enzymes include carbonic anhydrase (hydration of carbon dioxide in red blood cells), carboxypeptidase A (pancreatic peptidase), NAD-dependent dehydrogenases (alcohol dehydrogenase in liver), leucine aminopeptidase (peptidase in kidney and gastric mucosa), pyruvate carboxylase (citric acid cycle component), and leukotriene A4 hydrolase (synthesis of lipid mediators in neutrophils) (White et al., Principles of Biochemistry, New York, New York, McGraw-Hill Book Company, 1973). Zinc also serves as an important structural component of many proteins such as DNA binding proteins in a structure commonly termed "zinc fingers"(Schultz et al., Textbook of Biochemistry with Clinical Correlations, pp. 91-134, Devlin (ed.), New York, New York, Wiley-Liss, 1992).

Zinc compounds, primarily zinc salts, have shown utility in a number of areas. Examples are wound healing (Agren, Acta. Derm. Venereol. Supp. (Stockh) 154:1-36, 1990; Pastorfide et al., Clin. Ther. 11:258-63, 1989), healing of gastric ulcers (Frommer, Med. J. Aust. 2:793-96, 1975), inhibition of leukotriene A4 hydrolase (prevention of the formation of lipid mediators of inflammation) (Wetterholm

et al., Arch. Biochem. Biophys. 311:263-71, 1994), and the inhibition of certain viruses such as human immunodeficiency virus (HIV) (Bridger et al., J. Med. Chem. 38:366-78, 1995), inhibition of the HIV protease (Zhang et al., Biochemistry 30:8717-21, 1991), herpes virus (Kumel et al., J. Gen. Virol. 71:2989-97, 1990; Fridlender et al., Virology 84:551-54, 1978; Gordon et al., Antimicrob. Agents Chemother. 8:377-80, 1975), vaccinia virus (Katz et al., Antimicrob. Agents Chemother. 19: 213-17, 1981; Zaslavsky et al., J. Virol. 29:405-48, 1979), foot and mouth disease virus (Firpo et al., Arch. Virol. 61:175-81, 1979), and rhino virus(Korant et al., J. Virol. 71:2989-97, 1976).

In addition to zinc(II) salts, a number of zinc(II) complexes have been made and characterized. However, in most instances such zinc(II) complexes have merely been studied to determine the coordination geometry of the metal, or luminescence thereof. For example, Jordan et al. (Inorg. Chem. 30:4588-93, 1991) report the structural dependence of the luminescence from bis(substituted benzenethiolato) (2,9-dimethyl-1,10-phenanthroline) zinc(II) coplexes, while Monge (Acta Cryst. B33:2329-31, 1977) reports the crystal structure of a (dicyanide)(2,9-dimethyl-1,10-phenan-throline) zinc(II) complex. Other researchers have reported zinc(II) complexes with 1,10-phenanthroline, but not for use as biologically active compounds (Fitzgerald et al., J. Chem. Soc. Dalton Trans. 141-49, 1985; Romero et al., Polyhedron 10:197-202, 1991; Bell et al. (Inorganica Chimica Acta. 156:205-11, 1989; Reimann et al., Inorg. Chem. 5:1185-89, 1966; Bencini et al., Inorg. Chem. 28:1963-69, 1989; Hu and Liu, Acta Cryst. C47:2326-33, 1991; Cremers et al., Acta Cryst. B36:3097-99, 1980).

While the use of zinc salts appear promising for use in certain therapeutic areas, there is still a need in the art for additional zinc-containing compounds, complexes or compositions which possess biological activity. The present

invention fulfills this need, and provides further related advantages.

Summary of the Invention

This invention is generally directed to zinc(II) complexes and methods relating thereto. More specifically, the zinc(II) complexes of the present invention comprise zinc(II) complexed by a multi-dentate ligand. For example, the invention is directed to a zinc(II) complex comprising zinc(II) complexed by neocuproine, wherein the ratio of neocuproine to zinc(II) is greater than 1:1 (preferably at least 2:1). As another example, the invention is directed to a zinc(II) complex comprising a zinc(II) complexed by a ligand selected from bathocuproine disulfonic acid and pharmaceutically acceptable salts thereof, preferably in ligand:zinc(II) ratio of 1:1 to 3:1.

The invention also provides for a zinc(II) complex comprising zinc(II) complexed by a multi-dentate ligand, wherein the multi-dentate ligand is not 1,10-phenanthroline, neocuproine, bathocuproine disulfonic acid and pharmaceutically acceptable salts of bathocuproine disulfonic acid.

Another aspect of the invenition is a composition comprising a zinc(II) complex and a pharmaceutically acceptable carrier or diluent, wherein the zinc(II) complex comprises zinc(II) complexed by a multi-dentate ligand, and wherein the ratio of the multi-dentate ligand to zinc(II) ranges from 1:1 to 3:1.

A further aspect of the invention is a composition comprising a zinc(II) complex as described above in combination with a pharmaceutically acceptable carrier or diluent.

Still another aspect of the invention is the use of a zinc(II) complex as an active therapeutic agent. The zinc(II) complex may be any of the complexes described above.

The invention also provides for the use of a zinc(II) complex for the manufacture of a medicament, where an appropriately manufactured medicament can be used for treating inflammation in a warm-blooded animal, inhibiting viral replication in a warm-blooded animal or for inhibiting the transmission of a sexually transmitted disease in a warm-blooded animal. In a preferred embodiment, the medicament is formulated for topical administration, for example, topical administration to the epithelium of the vaginal mucosa, cervix, anus or penis.

The invention additionally provides a method for treating inflammation in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex. As an additional embodiment, the invention provides a method for inhibiting viral replication in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex. As a still further embodiment, the invention provides a method for inhibiting the transmission of sexually transmitted diseases in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex.

The zinc(II) complexes of the invention have utility for the inhibition of lipid mediators of inflammation, and for inhibiting viral activity and infection, including (but not limited to) HIV replication in an HIV-infected animal. Methods of the present invention comprise administering to an animal in need thereof an effective amount of a zinc(II) complex.

Other aspects of this invention will become evident upon reference to the attached figures and following detailed description. All references identified herein are hereby incorporated by reference in their entirety

Brief Description of the Drawings

Figure 1 is the x-ray crystal structure of a representative zinc(II) complex of this invention (i.e., zinc(II):neocuproine (2:1)).

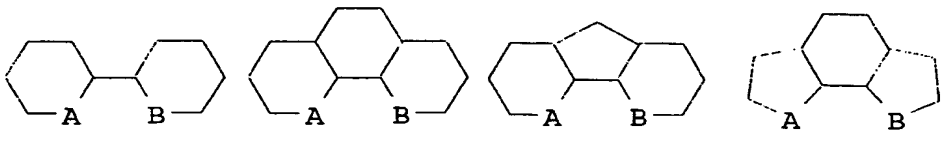
Figure 2 is the absorption spectrum of a further representative zinc(II) complex of this invention (i.e., zinc(II):bathocuproine disulfonic acid ("BCDS") (2:1)), and presents the absorption spectrum of BCDS for comparison.

Detailed Description

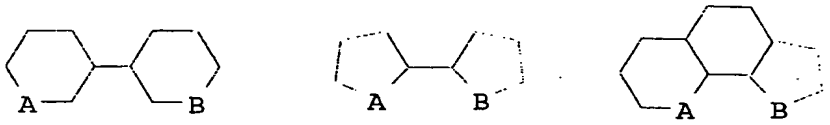
This invention is generally directed to zinc(II) complexes. Zinc is almost always found in the +2 oxidation state, in which it is invariably quite stable. (See, generally, Cotton and Wilkinson, Advanced Inorganic Chemistry, 5th ed., John Wiley & Sons, New York, pp. 503-527, 1988). Zinc(II) has previously been reported to have four coordination sites, and to generally possess a tetrahedral configuration.

In general, chelating agents are coordination compounds in which a single ligand occupies more than one coordination position of a metal ion. If the ligand occupies two coordination positions, it is considered a bi-dentate ligand; if more than two coordination positions are occupied by the ligand, it is considered a poly-dentate ligand (such as a tri-dentate ligand or a tetra-dentate ligand). As used herein, a "multi-dentate ligand" is a bi-, tri- or tetra-dentate ligand which occupies two, three or four coordination sites, respectively, of zinc(II).

Any multi-dentate ligand which chelates zinc(II) to yield a zinc(II) complex is suitable in the practice of this invention. However, in a preferred embodiment, the multi-dentate ligands of this invention are selected from the following general structures I through VII:



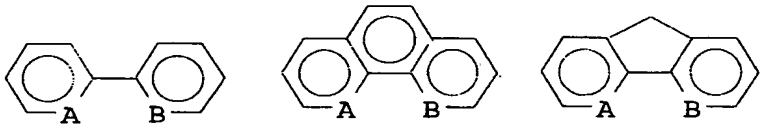
I II III IV



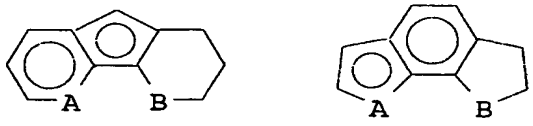
V VI VII

wherein A and B represent heteroatoms which may occupy coordination sites of zinc(II), and are preferably selected from nitrogen, oxygen, sulfur and phosphorous.

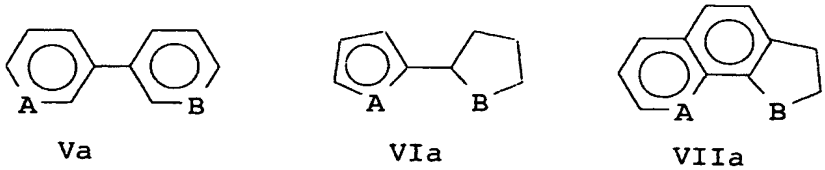
The rings of structures I through VII may be aromatic, non-aromatic or a mixture of both aromatic and non-aromatic rings. For example, the following structures are representative of such combinations:



Ia IIa IIIa



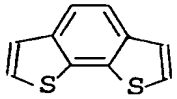
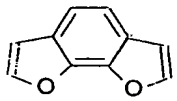
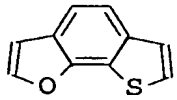
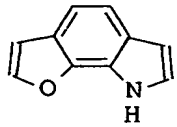
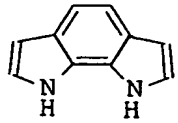
IIIa' IVa

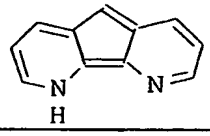
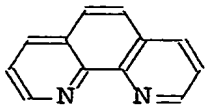
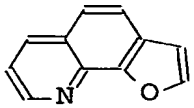
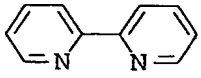


Va VIa VIIa

Representative examples of multi-dentate ligands of this invention having structures I through VII are set forth in Table 1. Specifically, Table 1 identifies the structure of the representative multi-dentate ligand, lists the corresponding chemical name, identifies the Chemical Abstracts Registration Number ("CA Reg. No."), and provides a corresponding reference (if available) describing the synthesis and/or chemistry of the identified multi-dentate ligand.

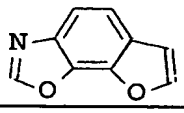
Table 1

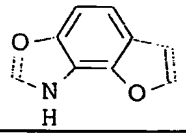
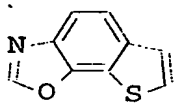
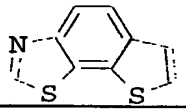
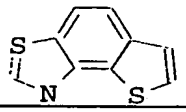
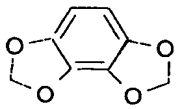
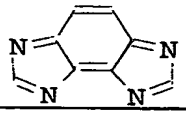
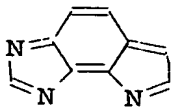
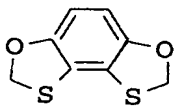
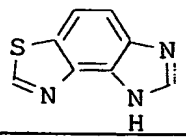
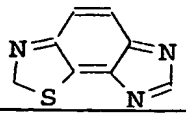
Structure	Name	CA Reg. No.	Reference
	benzo (2,1-b:3,4-b) dithiophene	211-53-0	Sturaro et al., Heterocycl. Chem. 27:1867, 1990
	benzo (2,1-b:3,4-b) difuran	211-47-2	Rene et al., Eur. J. Med. Chem.-Chim. Ther. 13:435, 1978
	thieno (3,2-g) benzofuran	438-31-9	Cagniant and Kirsch, Hebd. Seances Acad. Sci. C. 282:465, 1976
	2H-furo(3,2-g) indole	103671-62-1	Lawrence Jr., Eur. Pat. Appl. EP 173,520, 1986
	2H-benzo (2,1-b:3,4-b') dipyrrole	112149-08-3	Berlin et al., J. Chem. Soc. Chem. Commun. (15):1176, 1987

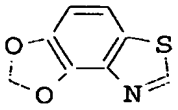
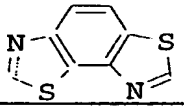
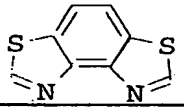
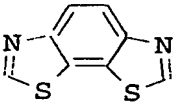
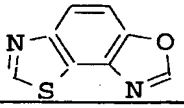
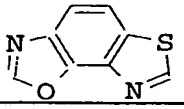
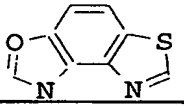
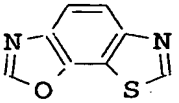
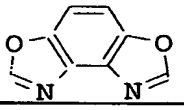
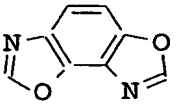
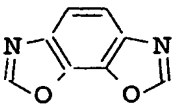
Structure	Name	CA Reg. No.	Reference
	1H-cyclopenta (2,1-b:3,4-b') bipyridine	42262-29-3	
	1,10-phenanthroline	66-71-7	
	furo (3,2-h) quinoline	234-28-6	
	2,2'-bipyridyl	366-18-7	

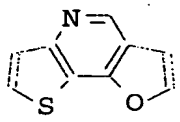
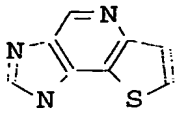
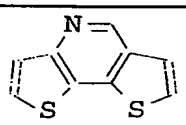
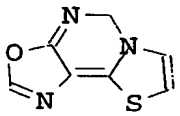
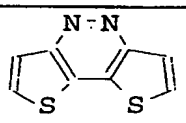
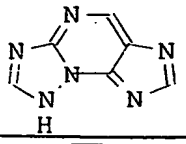
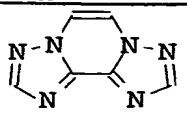
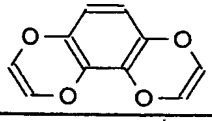
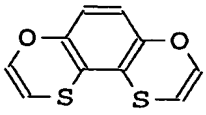
In structures I through VII above, further ring substitutions with heteroatoms are permitted. Preferably, such heteroatoms are selected from nitrogen, oxygen, sulfur, and phosphorus. For example, the compounds listed in Table 2 illustrate further representative multi-dentate ligands of the present invention having additional ring substitutions. As with Table 1, Table 2 identifies the structure of the representative multi-dentate ligands, lists the corresponding chemical name, identifies the CA Reg. No., and provides a corresponding reference (if available) describing the synthesis and/or chemistry of the identified multi-dentate ligand.

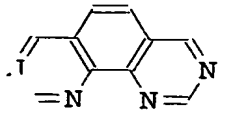
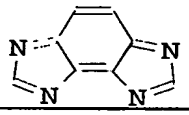
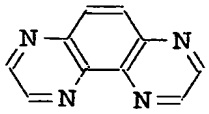
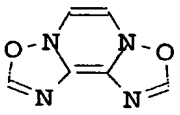
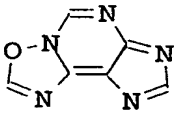
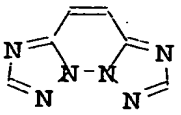
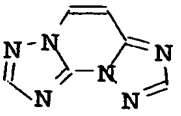
Table 2

Structure	Name	CA Reg. No.	Reference
	furano (3,2-g) benzoxazole	25885-39-6	

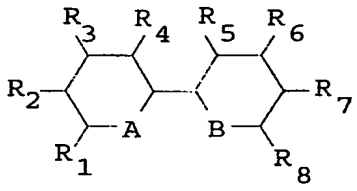
Structure	Name	CA Reg. No.	Reference
	furano (2,3-e) benzoxazole	66037-80-1	Turin et al., Fr. Demande 2,338,041, 1977
	thieno (3,2-g) benzoxazole	58188-85-5	Iddon et al., J. Chem. Soc., Perkin Trans. I 17:1686, 1975
	thieno (3,2-g) benzothiazole	72121-58-5	
	thieno (2,3-e) benzothiazole	211-36-9	
	benzo (1,2-d:3,4-d') bis (1,3) dioxide	211-50-7	Dallacker and Weiner, Justus Liebigs Ann. Chem. 725:99, 1969
	benzo (1,2-d:3,4-d') diimidazole	211-10-9	
	pyrrolo(2,3-e) benzimidazole	53068-46-5	Chetverikov et al., U.S.S.R. 425,906, 1974
	benzo (2,1-d:3,4-d') bis (1,3) oxathiole	211-54-1	
	2H-imidazo (4,5-e) benzothiazole	42341-40-2	
	2H-imidazo (4,5-g) benzothiazole	211-23-4	

Structure	Name	CA Reg. No.	Reference
	1,3-dioxolo (4,5-e) benzothiazole	77482-58-7	Foerster et al., Ger. Offen. 2,903,966, 1980
	benzo (1,2-d:3,4-d') bisthiazole	211-37-0	
	benzo (2,1-d:3,4-d') bisthiazole	23147-19-5	
	benzo (1,2-d:4,3-d') bisthiazole	10558-80-2	Grandolini et al., Ann. Chim. 58:91, 1968
	thiazolo(5,4-e) benzoxazole	211-35-8	
	thiazolo (5,4-g) benzoxazole	51273-21-3	
	thiazolo (4,5-e) benzoxazole	315-47-9	
	thiazolo (4,5-f) benzoxazole	67239-73-0	Fridman et al., Ikr. Khim. Zh. 44:399,1978
	benzo (2,1-d:3,4-d') bisoxazole	211-19-8	
	benzo (1,2-d:3,4-d') bisoxazole	211-20-1	
	benzo (1,2-d:4,3-d') bisoxazole	54935-19-2	Barker et al., J. Chem. Res. Synop. (9):328, 1986

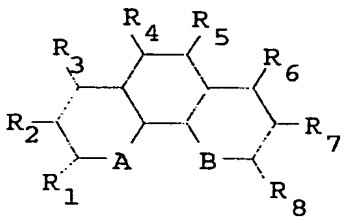
Structure	Name	CA Reg. No.	Reference
	furo (2,3-d) thieno (3,2-b) pyridine	110665-19-5	
	1H-imidazo (4,5-d) thieno (3,2-b)-pyridine	111163-54-3	Takada et al., Eur. Pat. Appl. EP 223,420, 1987
	dithieno (3,2-b:2',3'-d) pyridine	40826-38-8	Yang et al., Synthesis 2:130, 1989; Heeres et al., Syn. Commun. 2:365, 1972
	5H-oxazolo (4,5-e) thiazolo (3,2-c) pyrimidine	211-46-1	
	dithieno (3,2-c:2',3'-e) pyridazine	51974-92-6	Nonciaux et al., Bull. Soc. Chim. Fr. 12 Pt 2, 3318, 1973
	1H-(1,2,4) triazolo (5,1-b) purine	387-96-2	
	bis (1,2,4) triazolo (1,5-d:5',1'-c) pyrazine	55366-22-8	Vercek et al., Tetrahedron Lett. (51/52):4539, 1974
	benzo (2,1-b:3,4-b') dipyran	231-29-8	Monatsch 80:743, 1949
	benzo (1,2-b:4,3-b') bis (1,4)-oxathiin	231-34-5	

Structure	Name	CA Reg. No.	Reference
	benzo (1,2-e:3,4-e') dipyrazine		
	benzo (1,2-d:3,4-d') diimidazole	211-10-9	
	pyrazino (2,3-f) quinoxaline	231-23-2	Shim et al., Synthesis 2:116, 1980; Nasielski-Hinkins et al., J. Chem. Soc. Perkin Trans. 1:1229, 1975
	bis (1,2,4) oxadiazolo (2,3-d:3',2'-c) pyrazine	74382-83-5	
	(1,2,4)-oxadiazolo (3,2-i) purine	56248-95-4	Miura et al., Chem. Pharm. Bull. 23:464, 1975
	bis (1,2,4) triazolo (1,5-b:5',1'-f) pyridazine	51519-32-5	Polanc et al., J. Org. Chem. 39:2143, 1974
	bis (1,2,4) triazolo (1,5-d:1',5'-c) pyrimidine	76044-62-7	Brown and Shinozuka, Aust. J. Chem. 33:1147, 1980

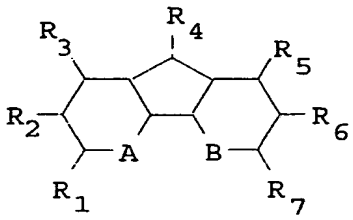
General structures I through VII identified above may possess further chemical moieties covalently attached to the structural backbone, as illustrated below:



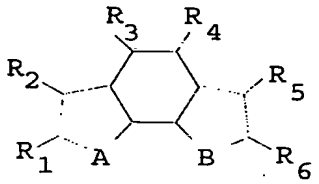
Ib



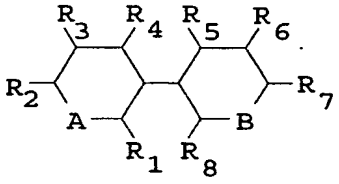
IIb



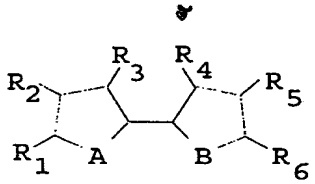
IIIb



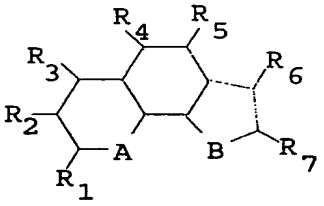
IVb



Vb



VIb

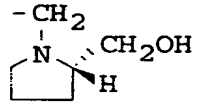
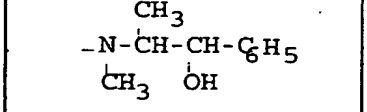
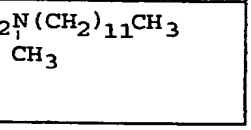


VIIb

wherein R_1 through R_8 are the same or different, and are selected from the following chemical moieties: -H, -OH, -X, -OX, -XOH, -COOH, -COOX, -CHO, -CXO, -F, -Cl, -Br, -I, -CN, $-NH_2$, -NHX, $-NX_2$, $-PX_2$, $-SO_3M$ (wherein M represents a pharmaceutically acceptable counterion for the sulfonate group ($-SO_3$), and may be H, Na, K, Cs, ammonium, etc.), $-SO_3X$,

$-PO_3H$, $-OPO_3H$, $-PO_3X$, $-OPO_3X$ and $-NO_2$. As used herein, "X" represents and an alkyl moiety or an aryl moiety. An "alkyl moiety" is a straight chain or branched, cyclic or noncyclic, saturated or unsaturated, substituted or unsubstituted carbon chain containing from 1-20 carbon atoms; and an "aryl moiety" is a straight chain or branched, cyclic or noncyclic, saturated or unsaturated, substituted or unsubstituted carbon chain containing at least one substituted or unsubstituted aromatic moiety and containing from 6-20 carbon atoms. Such chemical moieties may also be covalently attached to the ring fusion atoms. Representative examples of the chemical moieties of this invention include, but are not limited to, the moieties identified in Table 3 below.

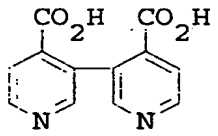
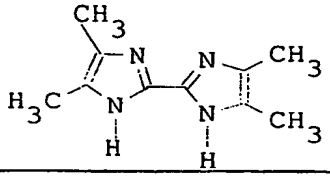
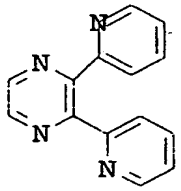
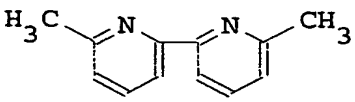
Table 3

$-H$	$-CH_3$	$-CH_2Br$
$-CH_2OH$	$-CH_2Cl$	$-CBr_3$
$-CH_2C_6H_5$	$-C_6H_5$	$-(CH_2)_{1-12}CH_3$
$-Cl$	$-CHO$	$-COOH$
$-COOMe$	$-CH=NOH$	$-CH_2NH_2$
$-CH_2C\equiv CH$	$-CH=CH_2$	$-P(C_6H_5)_2$
$-CH_2CH(CO_2H)_2$	$-CON(CH_2COOH)_2$	$-CH_2N(CH_2COOH)_2$
		
$-Ph-SO_3Na$		

Representative examples of the multi-dentate ligands possessing further chemical moieties covalently attached to the structural backbone of structures I through VII are presented in Table 4. In particular, Table 4 identifies the structure of the representative multi-dentate ligands, lists the corresponding chemical name, identifies the CA Reg. No., and provides a corresponding reference (if available)

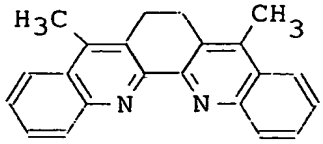
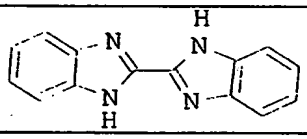
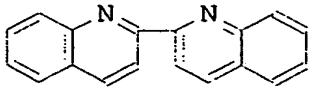
describing the synthesis and/or chemistry of the multi-dentate ligand.

Table 4

Structure	Name	CA Reg. No.	Reference
	2,2'-bipyridine-4,4'-dicarboxylic acid	6813-38-3	
	2,2'-bis (4,5-dimethyl imidazole)	69286-06-2	J. Organomet. Chem. 307:39, 1986
	2,3-bis (2-pyridyl) pyrazine	25005-96-3	(Aldrich: 28,164-16)
	5,5'-dimethyl-2,2'-bithiophene	16303-58-5	
	6,6'-dimethyl-2,2'-dipyridine	4411-80-7	Kauffmann et al., Chem. Ber. 109:3864, 1976

The chemical moieties covalently attached to the structural backbone may be joined to yield an aromatic or nonaromatic cyclic chemical moiety. Representative examples of such cyclic chemical moieties are set forth in Table 5, which identifies the structure of the representative multi-dentate ligands, lists the corresponding chemical name, identifies the CA Reg. No., and provides a corresponding reference (if available) describing the synthesis and/or chemistry of the multi-dentate ligand.

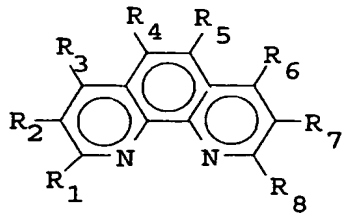
Table 5

Structure	Name	CA Reg. No.	Reference
	6,7-dihydro-5,8-dimethyl dibenzo (b)(1,10) phenanthroline	5298-71-5	
	bibenzimidazole	123067-51-6	
	2,2'-bisquinoline	119-91-5	(Aldrich: B3,540-7)

The synthesis of representative examples of the multi-dentate ligands of this invention are disclosed in Table 6 and Table 7 below. Specifically, in these tables the structure of the multi-dentate ligands are identified along with their CA Reg. No. and one or more references disclosing their synthesis and/or chemistry.

Table 6

Synthesis of Ligands for Representative Zinc(II) Complexes Having the Structure:



(R_2 through R_7 = hydrogen, unless indicated)

R_1	R_8	CA Reg. No.	Reference
$-CH_3$	$-CH_3$	484-11-7	O'Reilly et al., Aust. J. Chem. 13:145, 1960
$-CH_2Br$	$-CH_2Br$	78831-37-5	Weijen et al., J. Org. Chem. 57:7258, 1992; Jukkala et al., Helv. Chim. Acta. 75:1621, 1992; Chandler et al., J. Heterocycl. Chem. 18:599, 1981
$-CH_2Br$	$-CH_2OH$	142470-16-4	Weijen et al., J. Org. Chem. 57:7258, 1992
$-CBr_3$	$-CBr_3$		Chandler et al., J. Heterocycl. Chem. 18:599, 1981
$-CH_2Cl$	$-CH_2Cl$		Newkome et al., J. Org. Chem. 50:3807, 1985; Newcome et al., J. Org. Chem. 48:5112, 1983
$-CCl_3$	$-CCl_3$		Chandler et al., J. Heterocycl. Chem. 18:599, 1981; Newcome et al., J. Org. Chem. 48:5112, 1983
$-CN$	$-CN$	57709-63-4	Chandler et al., J. Heterocycl. Chem. 18:599, 1981; Sjoegren et al., Organometallics 11:3954, 1992
$-CH_2C_6H_5$	$-CH_2C_6H_5$	223-20-1	Sjoegren et al., Organometallics 11:3954, 1992
$-(CH_2)_{11}CH_3$	$-(CH_2)_{11}CH_3$		Menger et al., J. Am. Chem. Soc. 113:4017, 1991
$-(CH_2)_3CH_3$ (R_3=R_6=H, Ph	$-(CH_2)_3CH_3$	85575-93-5P	Sugihara et al., JP 02096578 A2, Jpn. Kokai Tokkyo Koho 113(15):132159v
$-(CH_2)_3CH_3$ (R_4=R_5=$-CH_3$)	$-(CH_2)_3CH_3$		Delton et al., EP 339973 A1, Eur. Pat. Appl. 112(21):19835p, 1989

R_1	R_8	CA Reg. No.	Reference
-Cl	-Cl	29176-55-4	Sjoegren et al., Organometallics 11:3954, 1992; Delton et al., EP 339973 A1, Eur. Pat. Appl. 112(21):19835p, 1989
$-CH_2OH$	$-CH_2OH$	78831-36-4	Chandler et al., J. Heterocycl. Chem. 18:599, 1981; Delton et al., EP 339973 A1, Eur. Pat. Appl. 112(21):19835p, 1989; Newcome et al., J. Org. Chem. 48:5112, 1983
-CHO	-CHO	57709-62-3	Ziessel, Tetrahedron Lett. 30:463, 1989; Toner, EP 288256 A2, Eur. Pat. Appl. 111(15):130322c; Bell et al., J. Inclusion Phenom. 5:149, 1987
-COOH	-COOH		Chandler et al., J. Heterocycl. Chem. 18:599, 1981
-COOMe	-COOMe		Chandler et al., J. Heterocycl. Chem. 18:599, 1981; Newcome et al., J. Org. Chem. 48:5112, 1983
-CH=NOH	-CH=NOH		Chandler et al., J. Heterocycl. Chem. 18:599, 1981
$-CH_2NH_2$	$-CH_2NH_2$		Chandler et al., J. Heterocycl. Chem. 18:599, 1981
-CHO	-H	33795-37-8	Toner, EP 288256 A2, Eur. Pat. Appl. 111(15):130322c
-COOH	-H	1891-17-4	Toner, EP 288256 A2, Eur. Pat. Appl. 111(15):130322c
$-CH_2C{\equiv}CH$	$-CH_2C{\equiv}CH$		Sjoegren et al., Organometallics 11:3954, 1992
$-C_6H_5$	$-C_6H_5$		Dietrich-Buchecker et al., Tetrahedron Lett. 23:5291, 1982
-Cl	$-CH_3$		Newcome et al., J. Org. Chem. 54:1766, 1989
$-CH=CH_2$	$-CH=CH_2$		Newkome et al., J. Org. Chem. 50:3807, 1985

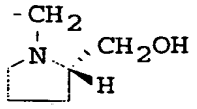
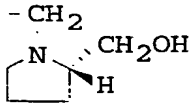
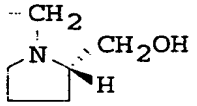
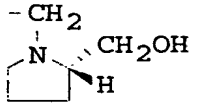
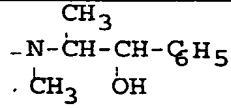
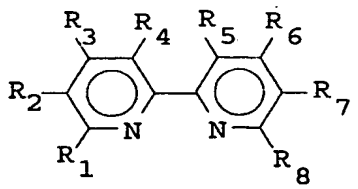
R_1	R_8	CA Reg. No.	Reference
$-P(C_6H_5)_3$	$-P(C_6H_5)_3$		Ziessel, Tetrahedron Lett. 30:463, 1989
$CH_2CH(CO_2H)_2$	$-CH_2CH(CO_2H)_2$		Newcome et al., Inorg. Chem. 24:811, 1985
$-CH_2N(CH_2)_{11}CH_3$ CH_3			Weijen et al., J. Org. Chem. 57:7258, 1992
			Weijen et al., J. Org. Chem. 57:7258, 1992
$-CH_2OH$			Weijen et al., J. Org. Chem. 57:7258, 1992
$-CH_2N(CH_2)_{11}CH_3$ CH_3			Weijen et al., J. Org. Chem. 57:7258, 1992
$-CH_2N(CH_2COOH)_2$	$-CH_2N(CH_2COOH)_2$		Mukkala et al., Helv. Chim. Acta 75:1621, 1992; Toner, EP 288256 A2, Eur. Pat. Appl. 111(15):130322c
$-CON(CH_2COOH)_2$	$-CON(CH_2COOH)_2$		Toner, EP 288256 A2, Eur. Pat. Appl. 111(15):130322c
$-CH_3$ (R_3=R_6= $-Ph-SO_3Na$	$-CH_3$	52698-84-7	Blair et al, Talanta 7:163, 1961

Table 7

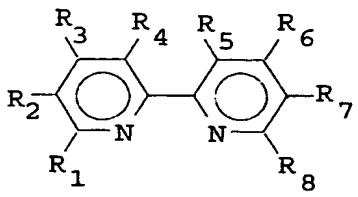
Synthesis of Representative Zinc(II) Complexes Having the Structure:



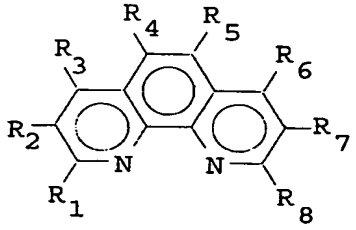
(R_2 through R_7 = hydrogen, unless indicated)

R_1	R_8	CA Reg. No.	Reference
-CN	-CN	4411-83-0	Sjoegren et al., Organometallics 11:3954, 1992
$-CH_2Cl$	$-CH_2Cl$	74065-64-8	Bell et al., J. Inclusion Phenom. 5:149, 1987
-CHO	-CHO		Newkome et al., J. Org. Chem. 50:3807, 1985
$-CH=CH_2$	$-CH=CH_2$		Newkome et al., J. Org. Chem. 50:3807, 1985
(R_1 and R_2= benzo moiety)	(R_7 and R_8 = benzo moiety)	119-91-5	(Aldrich: B3,540-7)

In one embodiment of this invention, the multi-dentate ligands are selected from the following structures:



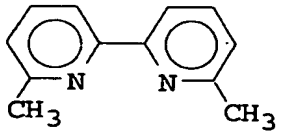
Ic



IIc

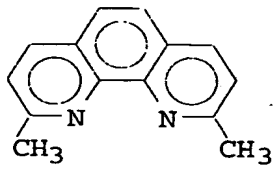
wherein R_1 through R_8 are the same or different, and are selected from hydrogen, an alkyl moiety and an aryl moiety.

In a preferred embodiment, the multi-dentate ligand is 6,6'-dimethyl-2,2'-dipyridine having structure Id:

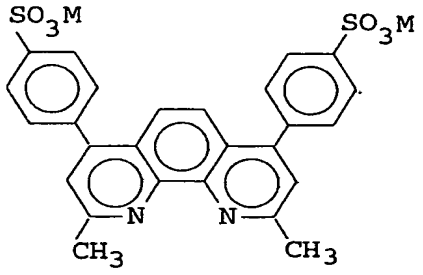


Id

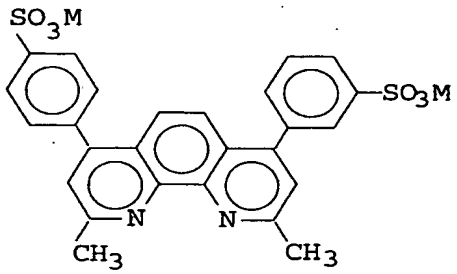
In a further preferred embodiment, the multi-dentate ligand is neocuproine (2,9-dimethyl-1,10-phenanthroline) having structure IId, or is bathocuproine disulfonic acid or a pharmaceutically acceptable salt thereof ("BCDS") having one of the isomeric structures IIe, IIe', IIe'' or IIe''' wherein M represents H or other counterion to form the pharmaceutically acceptable salt:



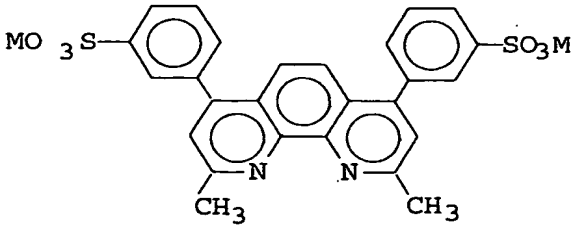
IId



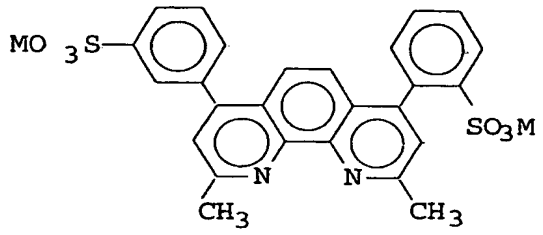
IIe (para,para)



IIe'(meta, para)



IIe'' (meta, meta)



IIe''' (ortho, meta)

Unless otherwise indicated, BCDS refers to a physical mixture of the above isomers (i.e., IIe, IIe', IIe'' and IIe'''). Typically, the ratio of the various isomers (i.e., IIe:IIe':IIe'') vary depending upon the commercial source of BCDS as follows: Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin) 9.1:38.6:41.2; Spectrum Chemical Manufacturing Corp. (Gardena, California) 8.5:39.7:45.2; GFS Chemicals (Columbus, Ohio) 8.4:38.5:45.3; Janssen Pharmaceutica (subsidiary of Johnson & Johnson) (Beerse, Belgium) 4.6-8.7:36.4-39.4:44.4-55.9; with the IIe''' isomer present in commercial sources in only trace amounts (i.e., typically about 1%).

As discussed above, zinc(II) complexes of this invention may be made by contacting a multi-dentate ligand with a zinc(II) source. The multi-dentate ligands may be obtained from commercial sources, or may be synthesized by known organic synthesis techniques from commercially available reagents. Preferably, water soluble multi-dentate ligands are complexed with the zinc(II) in aqueous solution, employing, for example, $Zn(NO_3)_2$ or $Zn(SO_4)$ as the zinc(II) source. Essentially any source of zinc(II) may be used in the invention, where the counterion in the zinc source may be considered, at least formally, as the counterion of the zinc atom in the zinc(II) complex of the invention. The counterion contributed by the zinc(II) source may be exchanged, after

formation of the zinc(II) complex, with any pharmaceutically acceptable counterion, according to ion exchange techniques that are well-known in the art. The resulting zinc(II) complex may then be recovered by evaporation of solvent to yield the zinc(II) complex. Alternatively, if the multi-dentate ligand is not readily soluble in water, zinc(II) complexes may be formed by the above procedure employing a suitable non-aqueous (e.g., organic) solvent.

In the practice of this invention, the ratio of the multi-dentate ligand to zinc(II) may be any ratio which results in a zinc(II) complex. Preferably, the ligand to zinc ratio is at least 1:1, and more preferably at least 2:1. In a further embodiment, the ligand to zinc(II) ratio ranges from 1:1 to 3:1 (including 2:1). Such zinc(II) complexes may be made by the procedures identified in the preceding paragraph by reacting the appropriate molar ratios of the multi-dentate ligand and the zinc(II) ion source.

In the case of zinc(II):neocuproine, the molar ratio of zinc to neocuproine is preferably in excess of 1:1, and more preferably at least 2:1.

When administered to an animal for therapeutic, prophylactic or cosmetic purposes, the zinc(II) complexes of this invention may be readily formulated by techniques known to those skilled in the art. (Preferred zinc(II) complexes of this invention are colorless, which is highly desirable for consumer appeal and acceptance.) For example, the zinc(II) complexes may be combined with one or more suitable carriers or diluents to yield a pharmaceutical preparation suitable for topical, oral or parenteral application. Such diluents or carriers, however, should not interact with the zinc(II) complex to significantly reduce the effectiveness thereof. Effective administration will preferably deliver a dosage of approximately 0.01 to 100 mg of the zinc(II) complex per kg of body weight.

Methods for encapsulating compositions (such as in a coating of hard gelatin) for oral administration are well

known in the art (see, e.g., Baker, Richard, Controlled Release of Biological Active Agents, John Wiley and Sons, 1986)(incorporated herein by reference). Suitable carriers for parenteral application (such as intravenous, subcutaneous or intramuscular injection) include sterile water, physiological saline, bacteriostatic saline (saline containing 0.9 mg/ml benzyl alcohol) and phosphate-buffered saline. The zinc(II) complexes may be topically applied in the form of liquids, containing acceptable diluents (such as saline and sterile water) or may be applied as lotions, creams or gels, containing additional ingredients to impart the desired texture, consistency, viscosity and appearance. Such additional ingredients are familiar to those skilled in the art and include emulsifying agents such as non-ionic ethoxylated and nonethoxylated surfactants, fatty alcohols, fatty acids, organic or inorganic bases, preserving agents, wax esters, steroid alcohols, triglyceride esters, phospholipids such as lecithin and cephalin, polyhydric alcohol esters, fatty alcohol esters, hydrophilic lanolin derivatives, hydrophilic beeswax derivatives, hydrocarbon oils such as palm oil, coconut oil, mineral oil, cocoa butter waxes, silicon oils, pH balancers and cellulose derivatives.

Topical administration may by accomplished by applying an amount of the preparation directly to the desired area. The required dosage will vary according to the particular condition to be treated, the severity of the condition, and the duration of the treatment. Preferably, when the zinc(II) complex is topically applied in the form of a lotion, cream or gel, the preparation may contain about 1% to about 20% of a penetration enhancing agent. Examples of penetration enhancing agents include dimethylsulfoxide (DMSO), urea and eucalyptol. In the case of a liquid preparations for topical application, the concentration of penetration enhancing agent (such as DMSO) may comprise about 30% to about 80% of the preparation.

The zinc(II) complexes of the present invention posses utility as anti-viral agents, and are particularly effective in the inhibition of the AIDS virus. Human acquired immunodeficiency syndrome or "AIDS" is a fatal disease for which there is presently no cure. The disease is believed to be caused by a virus known as the human immunodeficiency virus, commonly referred to as "HIV." The virus is transmitted by HIV-infected individuals through the exchange of bodily fluids. HIV infection results most commonly from sexual contact with an infected partner and the sharing among intravenous drug users of hypodermic syringes previously used by an infected individual. A pregnant HIV-infected mother may infect her unborn child by trans-placental transmission, and HIV-contaminated blood is a possible source of infection for individuals subject to blood transfusion.

HIV infection causes a suppression of the immune system. The immune suppression renders the infected individual vulnerable to a variety of opportunistic infections and conditions that are otherwise kept in balance by a healthy immune system. Fatalities result from HIV infection due to the inability of AIDS patients to respond to treatment of the opportunistic infections and conditions as a consequence of their compromised immune systems. Because the virus may often remain dormant, the manifestation of AIDS from HIV infection may take as long as ten years.

One approach to the treatment of AIDS has targeted the opportunistic infections or conditions which result from HIV infection. The treatment of such infections or conditions, however, is ultimately ineffective and, while prolonging the life of the infected individual, does not treat the underlying HIV infection. A second approach to the treatment of AIDS targets the cause of the disease itself. Because AIDS results from viral infection, it is believed that viral inactivation may ultimately provide a cure. (Materials which are capable of viral inactivation or inhibition are referred to herein as "antiviral agents.")

To understand the mode of action of antiviral agents in the treatment of AIDS, an understanding of the process of HIV infection is necessary. HIV chronically infects specific immune cells known as T-helper cells, which are required for normal immune response. The HIV infected T-helper cells serve as hosts to the virus and facilitate the reproduction of the virus (the process of viral reproduction is commonly referred to as "replication"). After HIV infection, the infected host cell eventually dies, the replicated HIV virus is released, and the infection spreads to additional cells. This cycle continues unabated, depleting the population of T-helper cells and, in time, weakens the immune system to the onset of AIDS symptoms. Because T-helper cells are continuously produced by the body, the population of these cells may be reestablished in the absence of further HIV infection. Therefore, the progression of HIV infection (and the subsequent onset of AIDS) may be arrested by the prevention or inhibition of viral replication, and antiviral agents capable of inhibiting or preventing the replication of HIV should be effective in the treatment of AIDS.

At the genetic level, HIV replication requires the insertion of viral deoxyribonucleic acid ("DNA") into the genome of the host cell. The genome of the host cell consists of the cell's own DNA, and is responsible for the synthesis of materials essential to the cell's own function and proliferation. Once the viral DNA is inserted into the host genome, the host facilitates replication of HIV. The inserted viral DNA is an enzymatic product derived from viral ribonucleic acid ("RNA") and the action of an enzyme known as HIV reverse transcriptase. Inhibition of HIV reverse transcriptase precludes the formation of viral DNA required for insertion into the genome of the host. Viral replication is prevented by the absence of viral DNA in the host cell genome. Antiviral agents which inhibit HIV reverse transcriptase are thus potential therapeutic drugs for treatment of AIDS.

Accordingly, in yet another embodiment of the present invention, antiviral agents are disclosed for inhibiting HIV replication, as well as methods relating to the administration thereof to an HIV-infected patient. The antiviral agents of this invention are the zinc(II) complexes discloses above, and the methods include administration of a therapeutically effective amount of a composition which includes a zinc(II) complex in combination with a pharmaceutically acceptable carrier or diluent. Although not limited by the following theory, the zinc(II) complexes of this invention may enhance transport of zinc(II) into HIV infected cells which, in turn, may inhibit or inactivate the HIV protease and thus inhibit the replication of HIV. As used herein, the term "HIV" includes the various strains of the virus (such as HIV-1 and HIV-2).

Administration of the zinc(II) complexes of the present invention may be accomplished in any manner which will result in a systemic dose of a therapeutically effective amount of the zinc(II) complex to an HIV-infected animal or patient (including human patients). For example, such administration may be by injection (intramuscular, intravenous, subcutaneous or intradermal), oral, nasal, or suppository applications. Typically, preparations of the present invention include zinc(II) complexes in solution for various forms of injection, or in preparations which are formulated for the sustained release of the zinc(II) complexes for oral, nasal, or suppository dosage application and generally include one or more inert, physiological acceptable carriers. As used herein, the term "effective amount" means an amount of the zinc(II) complex which inhibits HIV replication in the patient. Suitable dosages may range from approximately 0.01 to 100 mg of zinc(II) complex per kg body weight.

The zinc(II) complexes of this invention may be screened for their ability to inhibit HIV replication using known techniques. For example, HIV virus replication may be monitored using the Cytopathic Effect (CPE) assay disclosed by

Bergeron et al. (J. Virol. 66:5777-5787, 1992). In this assay, the degree of infection is monitored by the appearance of fused cellular membranes ("syncitium"). Alternatively, assays directed to activity of HIV protease may be employed. For example, the assays and techniques disclosed in the following references may be employed: Ashorn et al., Proc. Natl. Acad. Sci. U.S.A. 87:7472-7476, 1990; Schramm et al., Biochem. Biophys. Res. Commun. 179:847-851, 1991; Sham et al., Biochem. Biophys. Res. Commun. 175:914-919, 1991; and Roberts et al., Science 248:358-361, 1990.

Inhibition of viral replication by the zinc(II) complexes of this invention may also be due to inhibition and/or prevention of viral entry into a cell. With respect to HIV, for example, the zinc(II) complexes may prevent viral entry by interfering with CD4 receptor binding and membrane fusion. This may be illustrated by observing the inhibitory effect of a zinc(II) complex of this invention on syncytium formation using a virus-free, genetically engineered syncytium formation assay (Fu et al., J. Virol. 7:3818, 1993). This assay relies upon the molecular recognition of gp120, gp41 and the CD4 receptor to create syncytium. Inhibition of syncytium formation in this assay indicates that the zinc(II) complexes inhibit HIV replication by preventing viral entry, presumably by interacting with the viral proteins gp120 and gp41, and thus prevent and/or inhibit gp120 and gp41 function related to viral binding and membrane fusion. Thus, the zinc(II) complexes of the present invention have utility in preventing and/or inhibiting the spread of HIV to uninfected cells.

Accordingly, in this aspect of the present invention, zinc(II) complexes may be formulated in a manner suitable for application to, for example, the vaginal or rectal mucosa, as well as the penis. Suitable formulations include, but are not limited to, solutions, creams, gels, ointments, foams, suppositories and powders, and may include a variety of additional components such as lubricants, preservatives, carriers and diluents, as well as other active ingredients

such as spermacides. Such formulations contain a sufficient quantity of the zinc(II) complex, and are applied to the epithelium of the vaginal mucosa, cervix, anus and/or penis in an amount sufficient to prevent and/or inhibit viral transmission.

In this embodiment, the zinc(II) complexes of the present invention may also serve to prevent and/or inhibit the transmission of sexually transmitted diseases in addition to HIV, including human herpes virus and Hepatitis virus (as well as Chlamydia). The zinc(II) complexes of this invention may also have contraceptive activity.

The zinc(II) complexes of this invention, in addition to inhibiting HIV replication, may also inhibit replication of other viruses. Such viruses include, but are not limited to, human T-cell leukemia (HTLV) I and/or II, human herpes virus (HSV1 and 2), cytomegalo virus (human, hCMV, and murine, mCMV), encephalomyocarditis viruses (HAV, HBV, HCV (EMCV), Epstein Barr virus (EBV), human hepatitis virus (e.g., hepatitis B virus, HBV), Varicella Zoster virus, Rhinovirus, rubella virus, respiratory syncytium virus (RSV), influenza viruses A and B, parainfluenza viruses and adenovirus. One skilled in the art could readily assay the zinc(II) complexes of this invention for their inhibitory activity with regard to these viruses, as well as other viruses.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

The examples which follow illustrate the preparation, characterization and utility of certain exemplary embodiments of the zinc(II) complexes of the present invention. To summarize the examples that follow: Example 1 illustrates the synthesis and characterization of zinc(II):neocuproine (2:1); Example 2 illustrates the synthesis and characterization of zinc(II):BCDS (2:1); Example 3 discloses methods for

monitoring zinc (II) transport into cells by the zinc(II) complexes of this invention; Example 4 discloses methods for assaying the zinc(II) complexes of this invention for their ability to inhibit synthesis of lipid mediators of inflammation; and Example 5 discloses methods for assaying the zinc(II) complexes of this invention for their ability to function as antiviral agents.

Example 1

Synthetic Procedure for Zinc(II):Neocuproine (2:1) and Characterization Thereof

Neocuproine hydrate was used as received from Aldrich Chemical Company ("Aldrich"), having the following properties: mp161°C-163°C; ^{1}H NMR (500MHz, DMSO-d_6) δ 8.32 (2H, d, J = 8.2), 7.85 (2H, s), 7.60 (2H, d, J = 8.1), 2.79 (6H, s); ^{13}C NMR (125MHz, DMSO-d_6) δ 158.0, 144.6, 136.1, 126.4, 125.3, 123.1, 24.9.

A solution of neocuproine hydrate (4.53 g, 20.0 mmol) in methanol (20 mL) was added to a stirred solution of zinc nitrate hydrate(2.97 g, 10.0 mmol) in methanol (20 mL). A white precipitate formed immediately. This material was collected on a filter, washed with methanol, and dried under vacuum to give 5.66g (91%) of the monohydrate as a white solid. Recrystallization from aqueous ethanol yielded zinc(II):neocuproine (2:1) as clear plates: mp225.5°C-226°C; UV-vis λ_{max} (H_2O) 226nm (ε = 5,185$M^{-1}cm^{-1}$), 276nm (ε = 2,992), 298nm (ε = 1,196); MS *m/z* (relative intensity) 546 $(M(^{68}Zn)-(NO_3)^-)^+$ (7), 545 $(M(^{67}Zn)-(NO_3)^-)^+$ (5), 544 $(M(^{66}Zn)-(NO_3)^-)^+$ (10), 542 $(M(^{64}Zn)-(NO_3)^-)^+$ (15), 334 (30), 209 (100); HRMS calcd for $C_{28}H_{24}N_5O_3{}^{68}Zn$ (M^+ less NO_3) 546.1128, found 546.1108; calcd for $C_{28}H_{24}N_5O_3{}^{67}Zn$ (M^+ less NO_3) 545.1150, found 545.1136; calcd for $C_{28}H_{24}N_5O_3{}^{66}Zn$ (M^+ less NO_3) 544.1140, found 544.1119; calcd for $C_{28}H_{24}N_5O_3{}^{64}Zn$ (M^+ less NO_3) 542.1171, found 542.1138. Anal. calcd. for $C_{28}H_{26}N_6O_7Zn$:

C, 53.90; H, 4.20; N, 13.47. Found: C, 54.15; H, 3.99; N, 13.46.

A clear plate of zinc(II):neocuproine (2:1) was mounted on a glass pin with epoxy and transfered to the diffractometer in a nitrogen stream where collection was done at -90°C. The crystals remained clear with little deterioration. The crystal had approximate dimensions of 0.08mm x 0.36mm x 0.36 mm. Twenty five reflections in the range of 24-32 degrees in two-theta were found, and an orientation matrix was determined providing a unit cell with a volume of 2947 $Å^3$. Reduction of data was carried out by the program XCAD4 and all further work was performed using the PC version of Siemens SHELX. The Laue merging R factor was 1.5% for 504 equivalent reflections with a density of 1.37 with four molecules in the unit cell.

The structure was solved by direct methods, and agreed with the heavy metal location as given by a Patterson function, and the structure was determined from Fourier difference maps. One counter-ion nitrate was found, as well as one nitrate bonded to the zinc atom, giving a five-coordinate complex as illustrated in Figure 1. Structure determination parameters are summarized in Table 8.

Table 8

Structure Determination Summary

A.	Crystal Data	
	Empirical Formula	$C_{30}H_{32}N_6O_4Zn$
	Color; Habit	Clear Plates
	Crystal Size (mm)	0.08 x 0.36 x 0.36
	Crystal System	Monoclinic
	Space Group	P2(1)/n
	Unit Cell Dimensions	$\underline{a}$ = 11.385(2) Å $\underline{b}$ = 15.440(3) Å $\underline{c}$ = 17.475(3) Å β = 106.37(3)°
	Volume	2947.3(15) $Å^3$
	Z	4
	Formula Weight	606.0
	Density (calc.)	1.366 Mg/m^3
	Absorption Coefficient	0.878 mm^{-1}
	F(000)	1264
B.	Data Collection	
	Diffractometer	Enraf-Nonius CAD4
	Radiation	MoKα (λ = 0.71073 Å)
	Temperature (K)	183
	Monochromater	Highly oriented graphite crystal
	2θ Range	2 to 50°
	Scan Type	ω
	Scan Speed	Variable; 1.5 to 5.5°/min in ω
	Scan Range (ω)	0.06°
	Background Measurement	Stationary crystal and stationary counter at beginning and end of scan, each for 0.5% of total scan time
	Standard Reflections	2 measured every 2 hr.
	Index Range	$0 \le h \le 13$, $-1 \le k \le 18$, $-20 \le l \le 19$
	Reflections Collected	5634
	Indep. Reflections	4949 (R_{int}=1.85%)
	Observed Reflections	3419 ($F>4.0\sigma(F)$)
	Absorption Corrections	Semi-Empirical

C. Solution and Refinement

System Used	Siemens SHELXTL PLUS
Solution	Direct Methods
Refinement Method	Full-Matrix Least-Sqrs.
Quantity Minimized	$\sum w(F_o-F_c)^2$
Extinction Correction	X=-0.00018(12), where F* = $F[1+0.002XF^2/\sin(2\theta)]^{-1/4}$
Hydrogen Atoms	Riding model, fixed isotropic U
Weighting Scheme	$w^{-1} = \sigma^2(F) + 0.0035F^2$
No. Parameters Refined	434
Final R Indices (obs. data)	R = 4.59%, wR = 6.81%
R Indices (all data)	R = 7.47%, wR = 8.01
Goodness-of-Fit	1.05
Largest and Mean Δ/σ	1.515, 0.041
Data-to-Parameter Ratio	7.9:1
Largest Difference Peak	0.52 $eÅ^{-3}$
Largest Difference Hole	-0.50 $eÅ^{-3}$

Example 2

Synthetic Procedure for Zinc(II):BCDS (2:1) and Characterization Thereof

A stock solution of bathocuproine disulfonic acid disodium salt (BCDS) was prepared by dissolving 27.96 mg of bathocuproine disulfonic acid disodium salt (Aldrich) in 100 ml of HPLC-grade methanol. This solution was further diluted 1:10 in methanol for a final concentration of 0.0495 mM.

Stock $Zn(NO_3)_2$ was prepared by dissolving 1.0764 g of $Zn(NO_3)_2 \cdot 6H_2O$ (Aldrich) in 25 ml HPLC-grade methanol followed by two consecutive 1:10 dilutions in methanol for a final concentration of 2.274 mM.

Bathocuproine disulfonic acid disodium salt (2.0 mL, 0.0495 mM) was placed in a quartz cuvette equipped with a magnetic stir bar and the absorption spectrum was acquired. To the cuvette was added 55 ml of 2.274 mM $Zn(NO_3)_2$ in methanol (1.25 equivalents) using a microliter syringe. The absorption spectrum was acquired with a Hewlett Packard 8452A diode array spectrophotometer and then normalized to correct for the increase in volume.

A spectrum of methanolic $Zn(NO_3)_2$ was also acquired as a control. This spectrum did not show any absorbance at wavelengths greater than 260 nm (data not shown).

The spectra of bathocuproine disulfonic acid disodium salt and Zinc(II)-bathocuproine disulfonic acid disodium salt (normalized to the same concentration) are presented in Figure 2. These data indicates that the observed shift in λ_{max} and the corresponding increase in the extinction coefficient at the λ_{max} (at wavelengths greater than 260 nm) is due to complexation of the metal atom by bathocuproine disulfonic acid disodium salt.

Example 3
Enhanced Zinc(II) Transport Into Cells Utilizing Zinc(II) Complexes

Detection of metal ion transport into intact cells may be determined utilizing one or more suitable cell lines (such as RBL-1 cells available from American Type Culture Collection, Rockville, Maryland). Cells are cultured under established conditions with and without the addition of a zinc(II) complex of this invention. After 24 or 48 hours incubation with the zinc(II) complex of this invention, the culture media is withdrawn and the cells are harvested and washed several times with fresh media or phosphate buffered saline by centrifugation. The resulting cell pellet is then analyzed for total zinc(II) content by, for example, atomic absorption spectroscopy. Based on this procedure, administration of zinc(II) complexes of this invention are shown to enhance and/or facilitate zinc transport into cells.

Example 4
Inhibition of Synthesis of Lipid Mediators of Inflammation

Inhibition of leukotriene synthesis can be conveniently shown by incubating enzymes or cell fractions containing enzymes with compounds which can be converted to leukotrienes by the action of these enzymes. For example, Leukotriene A_4 (LTA_4) hydrolase (also known as LTA_4 synthase) is involved in the formation of LTB_4 from LTA_4. Leukotriene A_4 (LTA_4) hydrolase is prepared as a crude fraction from rat lung tissue. The sodium salt of LTA_4 is used as a substrate and incubated with the enzyme fraction and a zinc(II) complex of this invention for 1 minute at 37°C. The reaction is terminated by the addition of ice cold methanol. The formation of LTB_4 is assessed by a specific RIA (Radmark et al., J. Biol. Chem. 259:12339-12345, 1984; Kuhl et al., Prostaglandins 31:1029-1048, 1986; Izumi et al., Biochem. Biophys. Res. Comm. 135:139-145, 1986).

The inhibition of the formation of lipid mediators of inflammation can also be determined in intact cells. This assay involves incubation of the rat basophilic leukemia cell line RBL-1 with the zinc(II) complexes of this invention, followed by radioimmunoassay (RIA) analysis for a prostaglandin product of the cyclooxygenase ($PGF_{2\alpha}$) and a leukotriene product of the 5-lipoxygenase (LTB_4). Positive and negative controls are also run in each assay (Boschelli et al., J. Med. Chem. 36:1802-1810, 1993). Using the above methodology, zinc(II) complexes of this invention are shown to inhibit the formation of lipid mediators of inflammation.

Example 5
Anti-Viral Activity

A. Inhibition of HIV

The zinc(II) complexes of this invention may be screened for their ability to inhibit human immunodeficiency virus (HIV) replication using known techniques. For example, HIV virus replication may be monitored using the Cytopathic Effect (CPE) assay disclosed by Bergeron et al. (J. Virol. 66:5777-5787, 1992). In this assay, the degree of infection is monitored by the appearance of fused cellular membranes ("syncitium"). Alternatively, assays directed to activity of HIV protease may be employed. For example, the assays and techniques disclosed in the following references may be employed: Ashorn et al., Proc. Natl. Acad. Sci. U.S.A. 87:7472-7476, 1990; Schramm et al., Biochem. Biophys. Res. Commun. 179:847-851, 1991; Sham et al., Biochem. Biophys. Res. Commun. 175:914-919, 1991; and Roberts et al., Science 248:358-361, 1990.

In another method, PHA-stimulated peripheral blood mononuclear cells (PBMC) are infected by HIV_{IIIB} in the presence of the zinc(II) complexes of this invention and cultured in the presence of the zinc(II) complexes of this invention for two weeks. The extent of HIV replication is assayed at 1 and 2 weeks by a p24 antigen capture ELISA assay. More specifically, PBMC are stimulated with PHA for 24 to 72 hours in basal medium, containing RPMI-1640, 10% fetal bovine serum, and 50 µg/mL gentamicin, and then cultured overnight in the presence of 250 units/ml IL-2. Treated PBMC are pelleted by centrifugation and resuspended to 0.75 x 10^6/mL in basal medium with appropriate dilutions of the zinc(II) complexes of this invention or with no zinc(II) complexes of this invention added (i.e., control). To each 0.5 mL aliquot of cells, 0.5 mL of appropriate HIV dilution is added. The virus-cell mixture is incubated for 2 hours at 37°C in a 5% CO_2 humidified atmosphere. Following the incubation period, the PBMC are

washed twice in phosphate-buffered saline. Cells are resuspended in 5 mL to 7 x 10^4 cells/mL in basal medium with (or without) the zinc(II) complexes of this invention. Each cell aliquot is dispensed into four replicate wells of a 48 well tissue culture plate. Cells are fed twice a week with appropriate medium.

At one week and two week culture timepoints the extent of HIV replication is assayed by a p24 antigen capture assay kit (Coulter Corp., Hialeah, Florida). PBMC are treated with buffered detergent to release viral proteins. The cell extract is absorbed to immunoassay titer plates and p24 is detected by binding of a monoclonal anti-p24 antibody coupled to an enzyme. Following the addition of a chromogenic substrate, the amount of p24 is quantified spectrophotometrically.

B. Inhibition of Other Viruses

Inhibition of other viruses by the zinc(II) complexes of this invention may be and/or were assayed by the techniques as described below, or techniques very similar thereto:

1. ADV (Adenovirus Type 5)

ADV, a member of the Adenoviridae family, causes respiratory pathology in humans.

ADV was assayed by the same Neutral Red technique as for HCMV, RSV, and MV, described below, only that A549 cells were used in the assay.

2. HBV (Hepatitis B Virus)

A member of the Hepadnavirus group, HBV causes hepatitis and liver cancer.

The HBV virions released by the cells were quantitated (Korba and Milman, Antiviral Res. 19:55, 1992) via DNA hybridization to specific radiolabeled HBV DNA fragments. In this technique, the EC_{90} was determined as the effective concentration of the drug which reduces the yield of HBV DNA by 90%. The CC_{50} was determined for the drug on uninfected cells, and the SI is the ratio CC_{50} / EC_{90}.

3. HCMV (Human Cytomegalovirus)

A member of the Herpesvirus group, HCMV causes fetal damage. HCMV is generally benign unless immunosuppression or immunodeficiency is present, as in AIDS or bone-marrow transplant patients in which retinopathy/blindness and marrow rejection occur respectively.

The same Neutral Red technique was used for HCMV as for RSV, MV, PIF-3 and the influenza viruses, except that the cells used were HFF.

4. HSV-1 (Herpes Simplex Type 1)

A member of the Herpesvirus group, HSV-1 causes cold sores and other oral pathologies.

Same technique as HCMV: HFF Cells, Neutral Red for Both EC_{50} and CC_{50}, with ratio of CC_{50}/ EC_{50} = SI

5. HSV-2 (Herpes Simplex Type 2)

A member of the Herpesvirus group, HSV-2 causes genital infections and possibly cervial/uterine cancer.

Same technique as HCMV; HFF Cells, Neutral Red for Both EC_{50} and CC_{50}, with ratio of CC_{50} / EC_{50} = SI

6. IF-A (Influenza A Virus)

The MDCK cell line was used with the same Neutral Red technique as for MV, PIF-3 and RSV.

7. IF-B (Influenza B Virus)

The same technique was used to determine the antiviral effects as with IF-A virus.

8. MV (Measles Virus)

A member of the Paramyxovirus group, MV causes measles in humans.

CV-1 cells were cultured in monolayer, and the cytopathic effect (CPE) of the virus was quantitated by vital dye (Neutral Red Uptake). This quantitative method was used for the determination of the CC_{50} also. The point of 50% dye uptake was determined for both the infected (EC_{50} and the uninfected CC_{50} cells), and the ratio determines the SI.

9. PIF-3 (Parainfluenza Type 3 Virus)

MA-104 cells were used with this virus and the same Neutral Red technique for determining EC_{50}, CC_{50}, and SI was used as was used for MV.

10. RSV (Respiratory Syncytium Virus)

A member of the Paramyxovirus group, RSV causes respiratory disease in children and elderly.

MA-104 cells, and the same Neutral Red Assays were used for RSV as was used for MV and PIF-3.

11. VZV (Varicella Zoster Virus)

VZV, a member of the Herpesvirus group, causes chickenpox and shingles in humans.

HFF cells were maintained in monolayer culture. VZV infections were measured by the plaque reduction technique, in which the virus was suspended in an agarose and distributed over the monolayer of HFF. Areas of destruction (plaques) were determined by removing the agarose and staining the remaining monolayer. Virus yield (plaque forming units) was calculated, and the effect of the test drug is reported as EC_{50}, the concentration which reduces viral yield by 50%. Cytotoxicity (CC_{50}) for this assay was determined by neutral red (vital dye) uptake on confluent HFF monolayers or by counting cells in rapidly proliferating HFF cells.

12. YFV (Yellow fever virus)

YFV, a member of the Flavivirus family, causes severe systemic fever and death in humans. Representative surrogate virus for Hepatitis C Virus (same family), which causes liver failure and liver cancer in humans.

YFV was assayed for prevention of cytopathic effect (CPE) on Vero Cells by a method similar to the Neutral Red technique. The method was nearly identical with the exception that cell viability was quantitated with a reagent called MTT (used in the original PC1250 HIV studies and referenced therein). This reagent measures directly the activity of the electron transport chain of mitochondria, and thereby the

health of the cells. Neutral Red measurements rely on the overall ATP levels of cells, as a function of viability.

In the above examples, the abbreviations used are as follows:

A549 - Human lung carcinoma cells cultured in vitro.

CC_{50} - Cytotoxic Concentration$_{50}$, the concentration of a drug which produces 50% loss of viability, used to describe the cytotoxic or deleterious potential of a drug (also referred to as IC_{50} or Inhibitory Concentration$_{50}$);

CV-1 - African Green Monkey Kidney Cells cultured in vitro;

EC_{50} - Effective Concentration$_{50}$, concentration of a drug which causes a half-maximal biological response (i.e., antiviral effect - the concentration of test substance which restores the culture to 50% viability);

EC_{90} - Effective Concentration$_{90}$, concentration of a drug which produces a 90% maximal biological response (for antiviral activity, the concentration which restores the culture to 90% viability);

HFF - Human Foreskin Fibroblasts, primary fibroblast cultures derived from newborn circumcision skin samples, cultured in vitro;

MA-104 - Embryonic Rhesus Monkey Kidney Cells cultured in vitro;

MDCK - Madin-Darby Canine Kidney Cells cultured in vitro;

SI - Selectivity Index, used to compare compounds with respect to a therapeutic potential by dividing the EC_{50} by either the CC_{50} or the CC_{90}.

Vero - Kidney cells derived from African Green Monkey and used for growing and testing a number of hemorrhagic fever causing viruses; and

2.2.15 - Specialized cells for expression of HBV (Korba and Milman, Antiviral Res. 15:217, 1991), 2.2.15 cells are a derivative of Hep G2 Cells (Human hepatocellular carcinoma) which is engineered with a plasmid containing tandem copies of

HBV genomes (Sells et al., PNAS 84:1005, 1988; and Sells et al., J. Virology 62:2836, 1987).

The data set forth in Table 9 was obtained according to the above-described methods using zinc(II):BCDS complex prepared as described in Example 2.

In Table 9, the H9 and ME180 cells system represents a activity of zinc(II):BCDS complex, in that it measures the transfer of HIV-1 (Strain SK1), from H9 cells (human lymphocyte cell line) to ME180 cells (human cervical epithelium cell line). This is a model for testing a compound for its utility at preventing sexual transmission of HIV. The SI for this assay is calculated by dividing the CC_{50} by the EC_{50}.

Table 9
Inhibition of Pathological Human Viruses

Virus	Cell	EC_{50} (μM)	CC_{50} (μM)	SI
HIV	H9 & ME180	5	>5000	>1000
HIV	CEM	64.4	>500	>7.7
VZV	HFF	82.5	>100	>1.2
MV	CV-1	14	>250	>17.9
RSV	MA-104	16	>250	>15.6
HCMV	HFF	41.9	>100	>2.4
HSV-2	HFF	26.2	>100	>3.8
HSV-1	HFF	17.4	>100	>5.7
HBV	2.2.15	137	>300	>2.2
ADV	A549	100	>250	>2.5
YFV	Vero	53	>1000	>19

The zinc(II) complexes of this invention inhibit viral replication in one or more of the above assays, and thus function generally as antiviral agents, and more specifically as antiviral agents to a specific virus. While specific pathogenic viruses are disclosed above for purposes of illustration, other viruses may be assayed by one skilled in the art by known techniques.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described

herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not to be limited except as by the appended claims.

Claims

1. A zinc(II) complex comprising zinc(II) complexed by neocuproine, wherein the ratio of neocuproine to zinc(II) is greater than 1:1.

2. The zinc(II) complex of claim 1 wherein the ratio of neocuproine to zinc(II) is at least 2:1.

3. A zinc(II) complex comprising zinc(II) complexed by a ligand selected from bathocuproine disulfonic acid and pharmaceutically acceptable salts thereof.

4. The zinc(II) complex of claim 3 wherein the ratio of ligand to zinc ranges from 1:1 to 3:1.

5. A zinc(II) complex comprising zinc(II) complexed by a multi-dentate ligand, with the proviso that the multi-dentate ligand is not selected from 1,10-phenanthroline, neocuproine, bathocuproine disulfonic acid and pharmaceutically acceptable salts of bathocuproine disulfonic acid.

6. The zinc(II) complex of claim 5 wherein the ratio of the multi-dentate ligand to zinc(II) ranges from 1:1 to 3:1.

7. A composition comprising a zinc(II) complex and a pharmaceutically acceptable carrier or diluent, wherein the zinc(II) complex comprises zinc(II) complexed by a multi-dentate ligand, and wherein the ratio of the multi-dentate ligand to zinc(II) ranges from 1:1 to 3:1.

8. A composition comprising a zinc(II) complex of claims 1, 3 or 5 in combination with a pharmaceutically acceptable carrier or diluent.

9. Use of a zinc(II) complex as an active therapeutic agent.

10. The use of claim 9 wherein the zinc(II) complex is a zinc(II) complex of claims 1 to 6.

11. Use of a zinc(II) complex for the manufacture of a medicament for treating inflammation in a warm-blooded animal.

12. Use of a zinc(II) complex for the manufacture of a medicament for inhibiting viral replication in a warm-blooded animal.

13. Use of a zinc(II) complex for the manufacture of a medicament for inhibiting the transmission of a sexually transmitted disease in a warm-blooded animal.

14. The use of claims 11-13 wherein the medicament is formulated for topical administration.

15. The use of claim 12 wherein the virus is selected from human T-cell leukemia I and/or II, human herpes virus, cytomegalo virus, encephalomyocarditis virus, Epstein Barr virus, human hepatitis virus, Varicella Zoster virus, Rhinovirus, rubella virus, respiratory Syncytium virus, influenza virus, parainfluenza virus and adenovirus.

16. The use of claim 13 wherein the sexually transmitted disease is of viral origin.

17. The use of claim 12 or 16 wherein the virus is human immunodeficiency virus.

18. The use of claim 13 wherein the medicament is formulated for topical administration to the epithelium of the vaginal mucosa, cervix, anus or penis.

19. A method for treating inflammation in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex.

20. A method for inhibiting viral replication in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex.

21. A method for inhibiting the transmission of sexually transmitted diseases in a warm-blooded animal, comprising administering to the animal an effective amount of a zinc(II) complex.

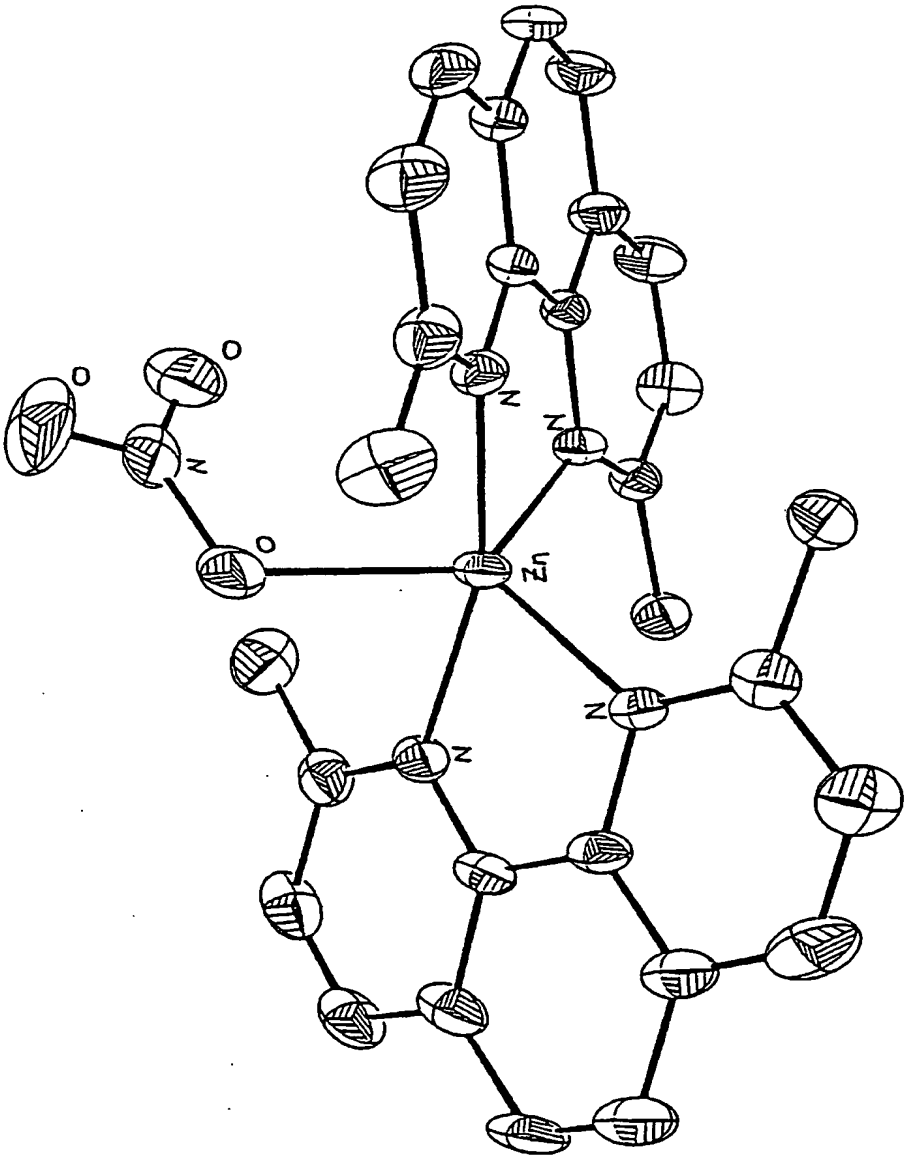


Figure 1

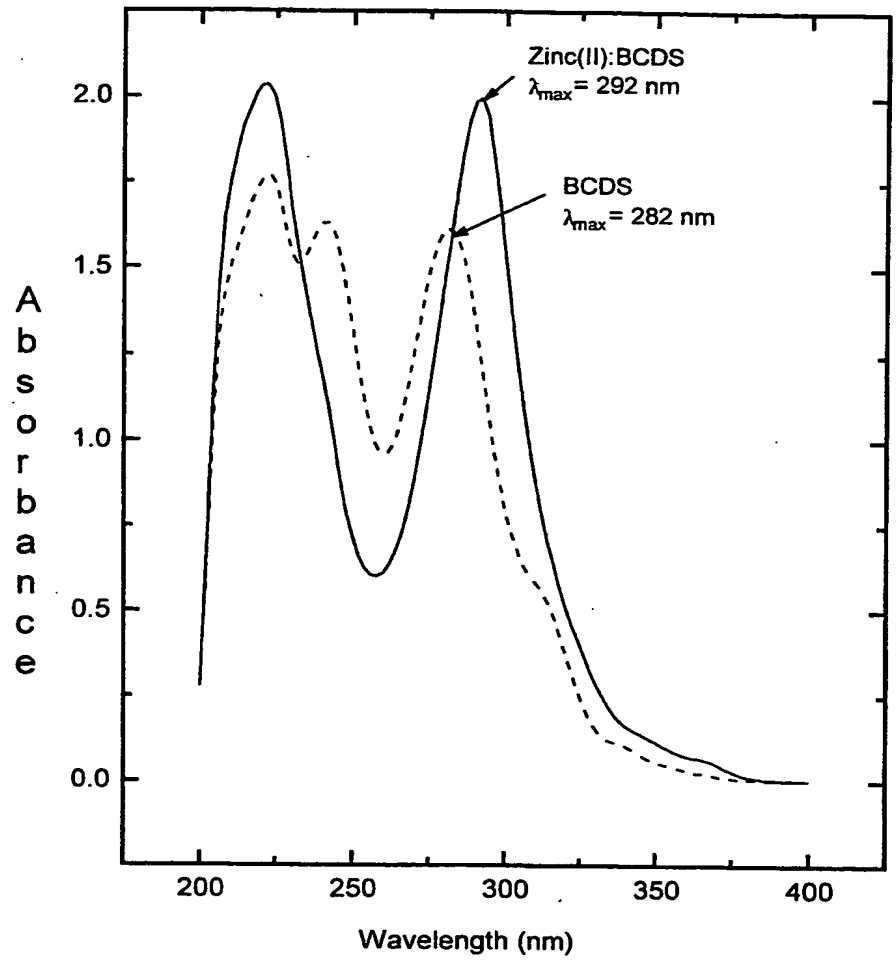

Zinc(II):BCDS
λ_{max}= 292 nm
BCDS
λ_{max}= 282 nm
Absorbance
2.0
1.5
1.0
0.5
0.0
200
250
300
350
400
Wavelength (nm)

Figure 2

INTERNATIONAL SEARCH REPORT

	International Application No PCT/US 96/11123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D471/04 A61K31/44 //(C07D471/04,221:00,221:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,956 241 (AUSTRALIAN NATIONAL UNIVERSITY) 22 April 1964 see page 2, line 110-page 3, line 96; page 5, code no. 8, 13; claim 1 ---	5,7
X	FR,A,2 250 529 (UNIVERSITY OF MELBOURNE) 6 June 1975 see claims 1,28; table I ---	5,7,13
A	WO,A,94 27594 (PROCYTE) 8 December 1994 see claims 1,8,15,16 --- -/--	5,11,15, 17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 1996	16.10.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Alfaro Faus, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ational Application No
PCT/US 96/11123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 116, no. 17, 1992 Columbus, Ohio, US; abstract no. 173435n, H. BOCK ET AL.: "Electron transfer and ion pairing" page 792; XP002015309 see abstract and Chemical Substance Index, page 10703, column 3, lines 29-31 & Z. NATURFORSCH., B: CHEM. SCI., , vol. 47, no. 2, 1992, pages 288-300, ---	5
X	CHEMICAL ABSTRACTS, vol. 102, no. 21, 1985 Columbus, Ohio, US; abstract no. 184485r, M. YAGI ET AL.: "Interactions of organic molecules with solvents and coexistents ions in the excited triplet states" page 527; XP002015310 see abstract and 11th Collective Index, Chem. Subst., page 71317, column 1, lines 64-71 & KENKYU HOKOKU, vol. 44-45, 1984, pages 59-69, ---	5
X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 89, no. 10, 1967, DC US, pages 2256-63, XP002015306 J.V. RUND ET AL.: "Ligand effects on the rate of metal-ion-catalyzed decarboxylation of dimethyloxaloacetic acid" see table I, 16-22 ---	5
X	INORGANIC CHEMISTRY, vol. 7, no. 5, 1968, EASTON US, pages 860-865, XP002015307 J.V. RUND ET AL.: "Electronic properties of some aromatic amine ligands and their abilities to enhance metal ion catalysis of the decarboxylation of beta-keto acids" see table II --- -/--	5

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/11123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INORGANIC CHEMISTRY, vol. 8, no. 1, 1969, EASTON US, pages 59-63, XP002015308 K. G. CLAUS ET AL.: "Neighboring-group effects on the rate of metal ion catalyzed decarboxylation of dimethyloxaloacetic acid" see table I -----	5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US96/11123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 19-21 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/compostion.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
On grounds of Articles 6 and 17.2a(ii) of the PCT (conciseness of claims) and of the Guidelines for Examination in the EPO, Part B, Chapt. III, 2.2 (economic reasons) the search has been based on a generalization of the preparation examples disclosed in the description.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/11123

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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